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Mobilization and collection of peripheral blood progenitor cells for transplantation

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Abstract

Bone marrow transplantation gradually expanded as a treatment modality for various malignant and non malignant disease conditions. Since the discoveries of the potential of Peripheral Blood Progenitor Cells (PBPC) in the hematopoietic reconstitution mid 1980s and early 1990s PBPC gradually replaced bone marrow as the preferred source of stem cells. The introduction of hematopoietic cytokines that can mobilize large number of progenitors into circulation accelerated PBPC usage. Technological advancements in the apheresis instrumentation greatly helped in the conversion from marrow to PBPC. PBPC collection is less painful, less expensive and transplant with PBPC results in faster hematological recovery than with marrow. Almost all of the autologous transplants are currently performed with PBPC and a similar trend is seen with the allogeneic transplants. The progenitor cell mobilization regimen for autologous patients can be cytokines alone or cytokines combined with chemotherapy. In the majority of the patients the required minimal cell dose of 2.5–5.0 $\times 10^6$/kg CD34+ cells can be collected in one or two apheresis collections. A few of autologous transplant patients who mobilize poorly require several collections. Allogeneic donors are generally mobilized with daily subcutaneous injections of G-CSF 10 μg/kg for 5 days. The PBPC are collected in one or two apheresis procedures. The side effects of G-CSF are generally mild to moderate; however rare serious reactions including rupture of the spleen have been reported. The collection of PBPC in pediatric patients poses additional challenges yet an adequate dose of cells can be collected with the available apheresis instrumentation. The apheresis collection procedures are safe with no serious adverse consequences. Future scientific advancements may expand the use of PBPC for other clinical application in addition to the current use for hematological reconstitution.

1. Introduction

Pioneering studies in animals in the 1940s and 1950s laid the foundation for hematopoietic cell transplantation in humans. The source of

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hematopoietic cells in these studies was bone marrow. The first attempt of a bone marrow transplant in humans in 1957, was unsuccessful. However it led to the discovery that large amounts of anticoagulated marrow could be infused into human patients without ill effects. A successful transplant with marrow from identical twins was reported in 1959 [1]. Bone marrow transplantation gradually expanded as a therapy to manage several malignant and non malignant disease conditions in the next two decades [2].

Stem cells were detected in the peripheral blood of mice in 1962 [3] and in humans in 1971 [4]. The development of apheresis instruments made it possible to collect a large number of progenitor cells from the peripheral blood [5]. However, interest in peripheral blood stem cell transplantation did not develop until mid 1980s. During 1985–1986 several centers from different parts of the world reported encouraging results of the autologous transplants using hematopoietic progenitor cells collected from peripheral blood [6–8]. The first allogeneic transplant with peripheral blood progenitor cells (PBPC) was reported in 1989 [9]. This transplant was performed because of a unique clinical situation where an HLA identical sibling donor of an 18 year old patient with acute lymphoblastic leukemia in third remission, was reluctant to undergo general anesthesia for bone marrow collection but willing to donate PBPC. The PBPC were collected from the unstimulated donor through ten apheresis procedures, and were extensively T cell depleted prior to infusion, to match a marrow graft. A bone marrow biopsy in the recipient on day 27 showed trilinage engraftment and patient died on day 32 because of other complications. Successful engraftment using allogeneic PBPC collected after mobilizing with G-CSF in a patient who had engraftment failure with bone marrow from the same donor was reported in 1993 [10]. Use of PBPC became more common after 1995 with the publication reports of successful allogeneic transplants with PBPC [11–13].

Currently, almost all of autologous and a majority of allogeneic transplants are performed with PBPC. Advantages of PBPC over bone marrow include (1) Elimination of the need of general anesthesia, pain and other side effects of bone marrow aspiration. (2) Patients with bone marrow metastases could be transplanted with autologous PBPC as there is a potential for tumor cell free collection [14]. (3) the hematological recovery with PBPC was faster then bone marrow significantly reducing the time to transfusion independence [15].

2. Mobilization of PBPC

Under steady state hematopoiesis the number of progenitor cells are low in circulation and several collections are required to obtain the required number of cells. A significant rebound increase in the number of stem cells in peripheral blood 2–3 weeks after myelosuppressive chemotherapy was reported in 1976 [16]. Transplant with chemotherapy mobilized cells successfully restored hematopoiesis following high dose therapy [17]. It was later found that there was a greater increase in hematopoeitic stem cells in the peripheral blood when chemotherapy was combined with growth factors [18].

3. Hematopoietic cytokines

Several exogenous hematopoeitic cytokines that can mobilize hematopoietic progenitor cells into circulation are now available. Granulocyte colony stimulating factor (G-CSF) (filgrastism) and granulocyte macrophage colony stimulating factor (GM-CSF) (sargramostim) are most commonly used for mobilization. The other cytokines studied include Thrombopoieten (TPO), FLT 3 Ligand, (FLT-3L), Stem Cell Factor and Interleukin-8 [19]. G-CSF was found to be more effective than GM-CSF in mobilizing progenitor cells in autologous transplant patients with different types of malignant diseases [20]. Several other factors also may have an effect on the degree of mobilization. These include the type of the growth factor used, the type of chemotherapy regimen used, pre- treatment and number of relapses. Poor mobilization could indicate a poor outcome in lymphoma patients undergoing autologous stem cell transplant [21]. A sufficient number of CD34+ cells to allow successful transplant can be obtained from the majority of patients [22].

4. Allogeneic PBPC mobilization

With the availability of growth factor to mobilize PBPC into circulation more allogeneic transplants are performed with PBPC. G-CSF is the growth factor of choice that is now used for mobilization in allogeneic donors. A dose of 10 μg/kg is given subcutaneously daily for 4–5 days and an adequate number of CD34+ cells can be collected by apheresis on day 5 and 6. Most side effects are mild to moderate include bone pain, headache, and fatigue [23]. Serious adverse effects have been recently reviewed include rare event of rupture of spleen, myocardial infarction and stroke [24]. Development of fatal [25] and serious [26] sickle cell crisis associated with G-CSF administration in individuals with sickle hemoglobin also have been reported.

The US National Marrow Donor Program criteria exclude donors with positive Hemoglobin solubility test (ex. Sickle Dex) from filgrastism—mobilized peripheral blood progenitor cell donation [27]. However, a recent prospective controlled study, comparing sickle cell trait subjects with normal subjects, found no adverse events in either group, when mobilized with 10 μg/kg filgrastism for 5 days. The median CD34+ cell yields were also similar [28]. Long term effects of G-CSF on healthy donors is not known. There were no obvious adverse effects in donors who had donated PBPC after G-CSF mobilization followed more than 3 years after donation [29].

5. Advantages of growth factor mobilized PBPC over bone marrow in allogeneic transplantation

The advantages to the donor with PBPC donation include avoidance of general anesthesia and pain and other adverse effects related to bone marrow collection. In a prospective randomized study comparing PBPC donation with bone marrow, PBPC products contained double the number of CD34+ cells, eight fold more of T and NK cells than bone marrow collection [30]. The advantages to the recipient were faster recovery of both neutrophil and platelets with PBPC compared to marrow without increasing risk of graft vs. host disease [31]. Another multi center study also reported faster neutrophil and platelet recovery but significantly more frequent acute and chronic GVHD in PBPC recipients than recipients of bone marrow cells. There were no significant differences in transplant related mortality, relapse rate and over all survival were found [32].

6. Cell dose

For a successful engraftment the PBPC product should contain adequate numbers of viable stem cells. There is no in vitro assay for "stem cells" therefore, other indirect assays are used. Most commonly mononuclear count, CD34+ cell counts and viability tests are performed as these can be performed quickly. The clonogenic assays (CFU and LT-CIC) are more time consuming. The engraftment is measured by recovery of absolute neutrophil count (ANC) of $>0.5 \times 10^9$/L and platelet count of $>20 \times 10^9$/L. Though the minimal dose of PBPC is not established most centers consider at least $2–2.5 \times 10^6$ CD34+ cells/kg as an acceptable collection [33].

A group of patients who received a dose between 1.17×10^6 and 2.48×10^6 kg CD34+ cells had prompt neutrophil recovery but approximately 10% had delayed platelet recovery [34]. The neutrophil recovery may also be influenced by the treatment regimen the patient received. Though increased CD34+ cell dose leads to faster neutrophil and platelet engraftment, increasing the cell dose above a certain threshold may have other complications particularly in allogeneic transplants. In HLA identical sibling transplantation with PBPC, infusion of higher dose $>8.3 \times 10^6$/kg CD34+ cells was associated with increased mortality from chronic GVHD. Efforts to accelerate neutrophil recovery with higher CD34+ cell dose must be counter balanced with the risk of chronic GVHD [35].

7. Timing of PBPC collection

The goal of PBPC collection is to collect an adequate number of CD34+ cells in as few apheresis

collection procedures as possible, therefore timing of the start of apheresis collection is important. In the autologous setting, patients respond differently to mobilization based on age, diagnosis, type of and duration of treatment. Several criteria have been recommended to select the optional time for collection. A peripheral blood WBC count can be readily obtained and many centers used a minimal count of 1.0×10^9/L as a point to start PBPC collection. A WBC count or mononuclear cell count obtained immediately prior to leukopheresis showed only weak correlation with the number of CD34+ cells collected with processing of three blood volumes. A good correlation was found between the absolute number of CD34+ cells on the day preceding leukopheresis procedure. With a peripheral blood CD34+ cell count of 20×10^6 cell/ml, 94% of the single apheresis collections performed the next day had a yield of $\geqslant 2 \times 10^6$ CD34+ cells/kg [36]. However, it may not be possible to obtain CD34+ counts quickly at some centers. A conventional WBC count differential on May-Giemsa stained peripheral blood smear was used to count circulating immature cells, (CIC) that included promyelocytes, myelocytes, metamyelocytes and erythroblasts. A two blood volume apheresis collection was performed when the peripheral blood CIC count was $\geqslant 1.0 \times 10^9$/L, yielded a CD34+ cell dose of $\geqslant 1 \times 10^6$ in 75% of the procedures [37]. Healthy allogeneic donors are usually mobilized with 10 μg/kg G-CSF injected subcutaneously for 5 days and apheresis collection started on day 5. Most donors mobilize well and an average targeted cell dose of 4–5×10^6 CD34+/kg can be collected with one or two apheresis procedures. One study found that administration of G-CSF in divided doses of 5 μg/kg twice daily leads to higher yield of CD34+ and fewer apheresis procedures, with no increased adverse effects [38]. Increased donor's age was inversely correlated with CD34+ cell yield, however poor mobilizers were found in all age groups [39]. The number of apheresis collections required to collect a targeted cell dose depends on the mobilization response and the collection efficiency. For autologous transplant patients CD34+ cell count is generally performed on each collection, and collections are continued until targeted cell dose is reached. Some rare patients with poor mobilization response may require up to 10 or more apheresis collections.

8. Collection of PBPC

There are several apheresis instruments available for the collection of PBPC. The collections are targeted to collect mononuclear cells. All the instruments are based on the principle of centrifugal force separation of anticoagulated blood into various component layers. The whole blood separates into distinct layers with increasing order by specific gravity into plasma, platelets, lymphocyte, granulocytes with red blood cells and mainly red blood cells. To collect PBPC the layer between granulocytes and platelets has to be harvested. These layers are not tightly compartmentalized therefore some cells on either side of the lymphocyte layer will also be collected. The question remains as to the nature and distribution of the sub population of progenitor cells close to the red cell bands and their effect on the clinical outcome. Generally the hematocrit of the PBPC collection is adjusted to be in the range of 2–3%. The collection of too many red cells may lead to complication in the recipient as red cells are hemolyzed and release free hemoglobin when cryo preserved PBPC are thawed for infusion. A large amount of free hemoglobin is toxic to the recipient and may impair renal function. Loss of large amounts of red cells in to the collection may also cause anemia in the donor. Platelets are another cellular component that will get collected into the product. Platelet loss has to be minimized as to avoid thrombocytopenia in the donor and to prevent clotting or clumping of the PBPC product.

9. Apheresis instruments

Many of the instruments that are used for the collection of other blood components including platelets and granulocytes can be used for PBPC collection. The instruments have computer controlled programs specific for each procedure and require specialized disposable collection kits.

9.1. Gambro

The Spectra (Gambro BCT, Lakewood, CO) is a dual needle access system with a loop type separation chamber. The Spectra has two programs for collection of PBPC. A single channel separation MNC program (versions 4.7, 5.1, 6.1, and 7.0) or a dual channel AutoPBSC program (Version 6.1). The MNC program is semi-automated, where in an operator can control the collection of the buffy coat fraction using a colored scale. The pumps are adjusted by the operator to maintain a salmon pink color in the collection line corresponding to a product hemotacrit of 2–3%. The Auto PBSC program is a fully automated collection procedure based on patient data entered at the start of the procedure.

9.2. Baxter

Baxter CS 3000 Plus (Baxter Biotech, Deerfield, IL) is a continuous flow centrifugal separation instrument with two serial separation chambers to isolate mononuclear cells. The next generation Baxter instrument Amicus collects MNC by employing a single chamber design, with the collection process occurring in stages. The MNC are collected intermittently into storage bags.

9.3. Fresenius

The Fresenius AS 104 (Fresenius USA, Walnut Creek, CA) utilizes a two stage separation chamber. In the first stage platelet and MNC rich plasma is produced and concentrated in the second stage. The COM.TEC (Fresenius Hemocare Bad Homburg, Germany) equipment has two programs, LP-MNC which works with one stage chamber and LP-PBSC-Lym program which works with a two-stage chamber [40].

9.4. Haemonectics

Haemonetics instruments MCS Plus (Haemonetics Corp., Braintree, MA) is an intermittent flow device using a bell shaped Latham bowel for component separation by centrifugation like a cream separator. Because of discontinuous flow it requires only a single venous access but the procedure time can be longer.

10. Comparison of instruments

All apheresis instruments can collect PBPC, but the differences are in the extra corporeal volume of the kits, single venous access vs. dual venous access, product volume, red cell and platelet loss and collection efficiency. With better collection efficiency, a required cell dose can be collected in a shorter period of time with lesser volume processed and in fewer numbers of procedures.

In a prospective randomized concurrent comparison of the COBE Spectra version 4.7, COBE Spectra Version 6 (Auto PBPC) and Haemonetics MCS+ there was no significant difference in the reduction of the platelet count following leukopheresis for PBSC collection. The absolute yield of CD34+ cells were lower with MCS+ compared with both COBE versions as the yields per unit volume of blood processed [41]. In a retrospective analysis one group found that the collection efficiency of COBE Spectra 4.7 was better than the CS 3000 Plus [42]. While in another retrospective analysis, it was found that the CS 3000 appears to be 8% greater CD34+ cell collection efficiency than the COBE Spectra 6.0 [43]. Platelet contamination in the PBPC products was lower with the Amicus than with the CS 3000 Plus. Amicus had a better CD34+ cell collection efficiency at lower peripheral blood WBC counts [44]. Fresenius COM.TEC LP-MNC program had better CD34+ cell collection efficiency but higher apheresis related platelet loss than LP-PBSC-Lym program [40].

11. Vascular access

Vascular access for apheresis collection procedures requires a continuous blood flow of 60–100 ml/min in adults. This could be established through the peripheral antecubital veins if they are large enough to accommodate a 16–18 gauge stainless steel needle for draw and at least a 19 gauge for the return. Whenever possible, it is

better to use peripheral venous access to avoid the complications associated with a central venous line placement. A stiff, dual lumen dialysis-type catheter placed in a subclavian, internal jugular or femoral vein can provide adequate flow. The femoral line can be placed at the bedside and has fewer placement related risk. However, it has a greater risk of infection and will limit the patient's mobility. The subclavian and jugular sites are good for long term catheter placement, but are associated with complications of pneumo- or hemothorax. Other catheter related complications include infection and thrombosis.

A majority of autologous transplant patients might already have a central venous access for chemotherapy purposes. However, small lumen ports may not be suitable for apheresis procedures. About 10% of allogeneic donors require a central venous access. In adult patients a 10–11.5 French catheter can provide adequate blood flow. While in children a 7–9 French can be used. Catheter patency is usually maintained with installation of heparin solution. Heparin-induced thrombocytopenia and thrombosis are rare but serious complications and some centers avoid the use of heparin. In a recent study among cancer patients undergoing apheresis collection for peripheral stem cells through central venous catheters there was no significant difference in catheter occlusion by thrombosis, whether the catheter was filled with heparin (100 u/ml saline) or normal saline [45]. At our center we flush the catheter only with saline.

12. Volume of blood processed

The number of PBPC collected with an apheresis procedure depends on the concentration of progenitor cells in the peripheral blood, volume processed and the collection efficiency of the instrument. The volume of blood processed in turn is influenced by the blood flow rate, processing time for volume targeted, and the capability of the patient/donor to tolerate the procedure. Conventionally two to three blood volumes are processed or the procedure is limited to a fixed period of time such as 4–6 h. There are several reports on the effects of large volume leukopheresis (LVL). The term LVL is not clearly defined and may vary from 15 L to 25 L [46]. Processing large volumes was required particularly in autologous transplant patients with low peripheral blood CD34+ cell counts, for the collection of adequate dose of CD34+ cells [47]. There was no significant decrease in the peripheral blood CD34+ cell count during three blood volume leukopheresis procedures in autologous transplant patients and the number of CD34+ cells collected was proportional to the volume of blood processed. The number of CD34+ cells collected after processing two blood volumes could be calculated for a value of peripheral blood CD34 count using a regression equation [48]. Adverse reactions with a LVL of 24 L with a mean collection time of 290 min was similar to those reported with conventional PBPC collections [46]. In G-CSF mobilized allogeneic donors, receiving prophylactic intravenous calcium during the collection procedure, CD34+ cells yields were similar when collection of PBPC were done in a single 25 L volume processed compared to the two 15 L volumes two consecutive days. There was less donor thrombocytopenia and inconvenience with a single 25 L apheresis collection [49].

13. Pediatric patients

Several malignant diseases in children may require high dose chemotherapy, followed by autologous hematopoetic stem cell transplant. PBPC collections in children weighing less than 25 kg has many social and technical challenges not faced in adult patients. These include venous access, anticoagulation, volume shifts and patient cooperation. All patients requires a central venous dual lumen catheter 7–9 Fr. Some centers use a combination of citrate and heparin or heparin alone for anticoagulation to minimize citrate effects [50]. At our center only citrate is used and ionized calcium levels are monitored pre apheresis and at intervals during the collection. The patients are encouraged to take oral calcium and are given intravenous calcium supplement based on the ionized calcium levels and patient symptoms. When the extra corporeal volume of the apheresis collection sets

exceed 10–15% of patient's blood volume, it can lead to volume depletion resulting in hemodynamic changes the patient could not tolerate. To minimize volume and red cell depletion the collection set is primed with compatible leukoreduced irriadiated CMV negative PRBC. The hematocrit of 42 day storage RBC will on average be 60%. It was the practice to dilute the RBC unit to about a 40% hematocrit when used for priming the set, because of concern that use of undiluted unit would result in a higher increase of hematocrit in the patient. In an experimental testing with a 1000 ml blood bag of 30% hematocrit simulating a 12–13 kg child, we found that priming the equipment with undiluted PRBC with hematocrit of 60% and avoiding rinse back at the end of the procedure will only increase the hematocrit in the patient by 2.5–4.5% and priming with RBC unit of 30% hematocrit will result in a decrease in hematocrit by 0–1%. Giving the patient back the first 50–100 ml rinse back should increase similar patient hematocrit by 0–2%, while causing 5–10% circulatory volume expansion [51]. Priming with undiluted PRBC will be beneficial, minimizing the potential risk of infection associated with the process of diluting the unit. Hydroxyethyl starch or 4% albumin was used to prime the equipment instead of blood for collections of PBPC in children with hemoglobin of 12 g/dl or more, when patients had lower hemoglobin they were transfused prior to PBPC collection procedures [52]. In a manual collection technique, described for children, 50 ml of blood was drawn into a bag with anticoagulant, and was then centrifuged. The buffy coat was transferred into a collection bag and the remaining blood was reinfused. These cycles were repeated until an adequate number of cells were collected [53]. However, with the currently available equipment, PBPC can be safely collected even from smaller children.

14. Anticoagulation

Citrate, ACD-A is the most commonly used anticoagulant in a ratio of 1:12 to 1:15 to prevent blood from clotting in the apheresis instrument during the collection of PBPC. Though the citrate is returned to the patient/donor it does not result in systemic anticoagulation due to metabolism and redistribution in the donor's circulation. The citrate binds with ionized calcium leading to a temporary decrease in the blood ionized calcium level. The citrate reaction symptoms include parasthesia, headache, light headedness, nausea, and chest tightness. In a majority of patients these symptoms are mild and may be relieved by oral calcium tablets or decreasing the blood flow rate in turn reducing the citrate infusion rate. In large volume leukopheresis ionized calcium and magnesium levels decreased by 35% and 56% respectively in inverse proportion to serum citrate levels [54]. Infusion of a calculated dose of calcium reduced the incidence of citrate related effects by 65–90% [54,55]. Potential side effects of calcium administration including risk of cardiac arrhythmias should be of concern. A careful individual, case by case, risk benefit analysis is recommended. Female patients with the blood volume less than 4.5 L may be ideal candidates for calcium administration [55]. Some centers use a combination of citrate and heparin or heparin alone to reduce the citrate toxicity. Heparin administration may also be associated with the adverse effect of bleeding.

15. Other apheresis complications

Red cell loss can occur when rinse back cannot be performed at the end of the PBPC collection procedure. A pre procedure hemoglobin of 10 g/dl should be maintained. There is some degree of platelet loss during PBPC collection. Depending on the pre procedure level, the platelet count will decrease 20–30% after the procedure. Each center may establish a protocol to include a minimal platelet count at which PBPC procedure could be started. If the platelet count is lower than the acceptable range, the patient may receive platelet transfusions. Low platelet counts are rare in allogeneic donors. In the US National Marrow Donor Program, if the platelet count after the first PBPC collection is $<80 \times 10^9$ L, the second PBPC product should not be collected [27].

PBPC collection procedures can have other, mostly reversible adverse effects, including

vaso-vagal reactions, pallor, diaphoresis, syncope, and hypotension. The adverse effects due to venipunture include hematoma, infiltration, nerve injury and arterial stick. Another rare complication is the hemolysis that can occur due to kinks in the lines of the collection kits. The adverse reaction rate for PBPC collection was reported to be 1.66% and there were no serious adverse consequences [56]. There is also a potential for a serious complication for air embolism. Current apheresis instruments have a built in safeguard to alarm when air is detected in the return line. There are no reports of cases of air embolism.

16. Summary

Most of the autologous and majority of allogeneic hematopoietic cell transplants are currently being performed with PBPC. For mobilizing progenitor cells into circulation a combination of chemotherapy and cytokines or cytokines alone is utilized for autologous collections while cytokines alone is used for allogeneic donors. Mobilization with G-CSF is relatively safe though there are rare serious adverse consequences. Peripheral blood CD34+ cell count of 20×10^6 cells/ml appears to be good indicator for selecting the optimal time start the apheresis collection. The adequacy of collection is assessed by product CD34+ cell count. The minimum number of cells considered necessary for prompt engraftment ($2.5–5.0 \times 10^6$/kg) can be collected by one or two apheresis procedures. The PBPC donations are a safe and less painful than bone marrow. Transplant with PBPC lead to faster hematologic recovery compared to bone marrow. Apheresis collection procedures are safe with no serious adverse consequences. The scientific advances in understanding the biology of the progenitor cells may lead to more clinical applications for the PBPC.

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